AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of the Claims

1. (Currently Amended) A method of assembling several DNA units in sequence in a DNA construct, which method comprises the steps of

a) providing each desired DNA unit to be assembled in the DNA construct, wherein each desired DNA unit has a restriction enzyme recognition sequence at its 5' end and a recognition sequence for the same restriction enzyme at its 3' end, said 3' recognition sequence being combined with ~~also comprising~~ a DNA methylase recognition sequence ~~that is compatible with such a restriction enzyme recognition sequence~~, and cleaving each desired DNA unit with said restriction enzyme, such that each desired DNA unit maintains said 3' DNA methylase recognition sequence,

b) providing a starting DNA construct having an accessible restriction site for said ~~the same or a compatible~~ restriction enzyme and cleaving the starting DNA construct with said restriction enzyme,

c) inserting a first desired DNA unit provided in step a) into the cleaved DNA construct, thereby generating a ligated product, and bringing the ligated product into contact with a DNA methylase such that the restriction site at the 3' end of the first desired DNA unit in the ligated product is abolished, thereby generating a ligated product containing a DNA modification,

d) cleaving the ligated product containing a DNA modification generated in step c) with ~~at an accessible unmodified recognition site for~~ said restriction enzyme such that said 5' restriction enzyme recognition sequence of said first desired DNA unit is cleaved,

e) repeating steps c) and d) with each subsequent desired DNA unit provided in step a), thereby generating a DNA construct containing all the desired DNA units in sequence.

e) inserting a next desired DNA unit provided in step a) into the cleaved ligated product from step d), thereby generating a next ligated product, and bringing the next ligated product into contact with a DNA methylase such that the restriction site at the 3' end of

the next desired DNA unit in the next ligated product is abolished, thereby generating a next ligated product containing a DNA modification,

f) cleaving the next ligated product containing a DNA modification generated in step e) with said restriction enzyme such that said 5' restriction enzyme recognition sequence of said next desired DNA unit is cleaved,

~~e)~~ g) repeating steps e) and f) ~~e) and d)~~ with each subsequent desired DNA unit provided in step a), thereby generating a DNA construct containing all the desired DNA units in sequence.

2. (Canceled)

3. (Previously Presented) The method of claim 1 wherein the methylase is a dam methylase of *Escherichia coli*.

4. (Currently Amended) A method of assembling several DNA units in a DNA construct which method comprises the steps of

a) providing each desired DNA unit to be assembled in the DNA construct, wherein each desired DNA unit has a *Xba*I recognition sequence 5'XXTCTAGA3', wherein XX is not GA, ~~(where XX is not GA)~~ at its 5' end and a *Xba*I recognition sequence 5'GATCTAGA3' at its 3' end,

b) providing a starting DNA construct having an accessible *Xba*I site and cleaving the starting DNA construct with *Xba*I,

c) inserting a first desired DNA unit provided in step a) into the cleaved DNA construct from step b), thereby generating a ligated product, and using the ligated product to transform a dam+ strain of *E. coli*, thereby generating a ligated methylated product,

d) recovering the ligated methylated product from step c) and cleaving the ligated methylated product at an accessible *Xba*I site with *Xba*I, such that said 5' *Xba*I restriction enzyme recognition sequence of said first desired DNA unit is cleaved, thereby generating a cleaved ligated product,

~~e) repeating steps c) and d) with each subsequent desired DNA unit provided in step a), thereby generating a DNA construct containing all the desired DNA units in sequence.~~

e) inserting a next desired DNA unit provided in step a) into the cleaved ligated product from step d), thereby generating a next ligated product, and using the next ligated product to transform a dam+ strain of *E. coli*, thereby generating a next ligated methylated product,

f) recovering the next ligated methylated product from step e) and cleaving the next ligated product at an accessible *Xba*I site with *Xba*I, such that said 5' *Xba*I restriction enzyme recognition sequence of said next desired DNA unit is cleaved,

g) repeating steps e) and f) with each subsequent desired DNA unit provided in step a), thereby generating a DNA construct containing all the desired DNA units in sequence.

5. (Currently Amended) The method of claim 1 or 3 ~~any one of claims 1 to 3~~, wherein the recognition sequences for the restriction enzyme and the DNA methylase ~~modification enzyme~~ are created in the DNA units prior to cutting with the restriction enzyme.

6. (Currently Amended) The method of any one of claims 1, 3, or 4 ~~claims 1 to 4~~, wherein the restriction enzyme recognition sequences ~~sites~~ are created in each DNA unit by means of a primer extension reaction.

7. (Currently Amended) The method of any one of claims 1, 3, or 4 ~~claims 1 to 4~~, wherein the DNA construct is an expression vector capable of facilitating expression of a protein encoded by the desired DNA units.

8. (Previously Presented) The method of claim 3, wherein the DNA modification of the ligated product containing a DNA modification is removed and the restriction site re-established by replicating the ligated product in a *dam*- strain of *E. coli*.

9. (Currently Amended) A method of making an assembly of several DNA units in sequence which method comprises the steps of:

a) providing a starting DNA construct comprising a first DNA unit with a recognition sequence for a first restriction enzyme at the 3' end of said DNA unit, and cleaving

said first DNA unit with said first restriction enzyme, thereby generating a cleaved starting DNA construct,

b) providing each desired DNA unit to be assembled in sequence, wherein each desired DNA unit has a recognition sequence at its 5' end for a second restriction enzyme which has a compatible ligation sequence with that of the first restriction enzyme, and a downstream recognition sequence for said first restriction enzyme followed by a downstream recognition sequence for a third restriction enzyme at its 3' end, and cleaving a first desired DNA unit with the second and third restriction enzymes, thereby generating a cleaved first desired DNA unit,

c) ligating said cleaved starting DNA construct with the cleaved first desired DNA unit generated in step b) to form a ligated product such that the ligation of the cleaved starting DNA construct and the cleaved first desired DNA unit abolishes the recognition site for the first restriction enzyme at a ligation junction of the cleaved starting DNA construct and the first cleaved desired DNA unit, and cleaving the ligated product with said first restriction enzyme, thereby generating a cleaved product,

~~d) repeating step b) with a subsequent desired DNA unit provided in step b) and ligating said subsequent desired DNA unit with the cleaved product from step c) to form a subsequent ligated product and cleaving the subsequent ligated product with said first restriction enzyme, and~~

~~e) repeating step d) with each desired DNA unit provided in step b) in turn so as to assemble the DNA units in sequence.~~

d) cleaving a next desired DNA unit provided in step b) with the second and third restriction enzymes, thereby generating a cleaved next desired DNA unit,

e) ligating the cleaved product from step c) with the cleaved next desired DNA unit from step d) to form a next ligated product such that the ligation of the cleaved product and the cleaved next desired DNA unit abolishes the recognition site for the first restriction enzyme at a ligation junction of the cleaved product and the cleaved next desired DNA unit, and cleaving the next ligated product with said first restriction enzyme, thereby generating a next cleaved product,

f) repeating step d) with a subsequent desired DNA unit provided in step b) and ligating said subsequent desired DNA unit with the next cleaved product from step e) to

form a subsequent ligated product and cleaving the subsequent ligated product with said first restriction enzyme, and

g) repeating step f) with each desired DNA unit provided in step b) in turn so as to assemble the DNA units in sequence.

10. (Currently Amended) A method of making an assembly of several DNA units in sequence which method comprises the steps of:

a) providing a starting DNA construct comprising a first DNA unit with a *Xba*I recognition sequence 5'TCTAGA3' at its 3' end, and cleaving ~~the~~ said first DNA unit with *Xba*I, thereby generating a cleaved starting DNA construct,

b) providing each desired DNA unit to be assembled in sequence, wherein each desired DNA unit has a *Spe*I recognition sequence 5'ACTAGT3' at its 5' end, and downstream *Xba*I recognition sequence 5'TCTAGA3' followed by a downstream *Sma*I recognition sequence 5'CCCGGG3' at its 3' end and cleaving a first desired DNA unit with *Spe*I and *Sma*I, thereby generating a cleaved first desired DNA unit, and dephosphorylating the 5' end of the cleaved first desired DNA unit, thereby generating a cleaved dephosphorylated first desired DNA unit,

c) ligating ~~the~~ said cleaved starting DNA construct with the cleaved dephophorylated first desired DNA unit generated in step b) to form a ligated product and cleaving the ligated product with *Xba*I, thereby generating a cleaved product,

~~d) repeating step b) with a subsequent desired DNA unit provided in step b) and ligating said subsequent cleaved dephosphorylated desired DNA unit with the cleaved product from step c) to form a subsequent ligated product and cleaving the subsequent ligated product with *Xba*I, and~~

~~e) repeating step d) with each desired DNA unit provided in step b) in turn so as to assemble the DNA units in sequence.~~

d) cleaving a next desired DNA unit provided in step b) with *Spe*I and *Sma*I, and dephosphorylating the 5' end of the cleaved next desired DNA unit, thereby generating a cleaved dephosphorylated next desired DNA unit,

e) ligating the cleaved product from step c) with the cleaved dephosphorylated next desired DNA unit from step d) to form a next ligated product and cleaving the next ligated product with *Xba*I,

f) repeating steps d) and e) with each desired DNA unit provided in step b) in turn so as to assemble the DNA units in sequence.

11. (Previously Presented) The method of claim 9 or claim 10 wherein the assembly occurs *via* stepwise addition of at least one DNA unit to a vector.

12. (Previously Presented) The method of claim 9 or claim 10 wherein the said first DNA unit is attached to a solid phase for use in step c).

13. (Previously Presented) The method of claim 12, wherein the solid phase is combined with a subsequent desired DNA unit in step c) to make several different assemblies.

14. (Previously Presented) The method of claim 9 or claim 10, wherein the recognition sequences in one or more of the DNA units are introduced by means of extension primers.

15. (Previously Presented) The method of claim 9 or claim 10, wherein the assembly of several DNA units is inserted into an expression vector which is used to transform a host capable of expressing a protein encoded by the assembly of several DNA units.

16. (Previously Presented) The method of any one of claims 1, 4, 9, or 10, wherein one or more of the DNA units encodes a catalytic or transport protein domain.

17. (Previously Presented) The method of claim 16 wherein one or more of the DNA units are derived from DNA sequences of polyketide synthesising enzyme domains.

18. (Withdrawn) The method of claim 16 wherein one or more of the DNA units are derived from peptide synthesising enzyme domain DNA sequences.

19. (Withdrawn) The method of claim 16 wherein one or more of the DNA units are derived from hybrid peptide polyketide enzyme domain DNA sequences.

20. (Withdrawn) The method of claim 16 wherein one or more of the DNA units are derived from fatty acid synthesizing enzyme domain DNA sequences.

21. (Previously Presented) The method of claim 16 wherein one or more of the DNA units encode modules comprising one or more catalytic or transport domains.

22.-48. (Canceled)